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HISTOPATHOLOGICAL EFFECTS OF BACILLUS THURINGIENSIS ON PIERIS BRASSICAE LARVAE

BY

Bahareth, O.M. and Sakr, S.A.

Biology Dept., Faculty of Applied Sciences,

Umm Al Qura University, Makkah, Saudi Arabia.

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ABSTRACT

The present work studied the effect of the bacterial insecticide, Bacillus thuringiensis on the 3rd and 5th larvae of Pieris brassicae. Treating larvae with a sublcthal dose of B.thuringiensis resulted in appearance of many external symptoms . The larvae became inactive and convulsions appeared in most of them after 24 hours of treatment. Histopathological changes appeared in the mid gut tissue, Malpighian tubules and nerve ganglion. The epithelial cells of the midgut as well as the Malpighian tubules showed marked cytoplasmic vacuolation and pyknotic nuclei. The nerve cell bodies exhibited an obvious destruction and many vacuoles appeared in the neuropile mass. The effects of B. thuringeiusis are similar in both the 3 rd and 5th larvae.

INTRODUCTION

The bacterial insecticide, Bacillus thuringienesis is widely used in control of many insects and lepidopteran larvae (Abdel-Fatah et al. 1977. Lacy et al . 1978, Knowden et al. 1993.) Bacillus thuringiensis was effective in reducing larval population of Diaphania indica on cucumbers (Schreiner, 1991). Lou et al. (1991) found that a new strain of B. thuringiensis (T M 13-14) isolated from dead larvae of Tenebrio molitor was toxic to several species of Lepidoptera, Diptera and Coleoptera. Bahareth and

Sakr (1994) reported that B. thuringiensis affected the survival and the number of heamocytes in 3rd and 5 th larvae of Masalia albida.

The larvae of the lepidopteran insect Pieris brassicae are considered to be one of the most important econmic pests. The rd and 5 th larvae were considered the most serious instars. They attack the plants, Coronopus squasmatas, Sinapis drvensis, Sisymbrium irio and copsella burspastori. The present work was aimed at studying the histological effects of the bacterial insecticide, Bacillus thuringiensis on the tissues of Pieris brassicae larvae.

MATERIAL AND METHODS

Third and fifth larval instars of Pieris brassicae were used in the present investigation. They were kept in clean reaing boxes and were allowed to feed on fresh lettuce.

The commercial bacterial insecticide Bacillus thuringiensis Known as SAN 415 (3200IU) was used. The test solutions were made up with dechlorinated tap water.

The 3 rd and 5 th larvae were divided into two groups : treated and controls. Those in the treated group were sprayed with a sublethal concentration of B. thuringiensis equivalent to 5 IU/g. body weight. For histological study, control and treated larvae were collected after 48 hours of treatment and fixed in Bouin's fluid. Larvae were then dehydrated in ascending series of ethyl alcohol, cleared in xylol and embedded in paraffin wax. sections of 4-6 microns thickness were cut and stained with hematoxylin and eosin.

RESULTS

I.External symptoms:

The earliest symptoms on poisoned larvae induced by B. thuringiensis were noticed after 12 hours. The treated larvae ceased feeding and became inactive after 24 hours, slight convulsions began to appear in most larvae; and after 48 hours, the

symptoms became severe. The treated larvae became wet with liquid that came out from the mouth and anus.

II.HISTOLOGICAL RESULTS:

1- Third larval instar:

a. control larvae.

The histological structure of the control 3rd larval instar of Pieris brassicae is seen in figure 1. The mid gut wall is made up of muscular coat to the outside consisting of two layers of muscle fibers, outer longitudinal fibers and inner circular ones. Next to the muscular coat inward, there is a basement membrane on which rest the cells of the epithelial layer (Fig. 2) The epithelium consists of two types of cells, the columnar and regenerative cells. The columnar cell is cylindrical, containing a large granular nucleus located near the middle portion of the cell. The regenerative cells are round or elongated cells, containing a large nucleus surrounded by basophilic cytoplasm. They are present in groups between the columnar cells. Many strands of fat bodies are observed. The fat body strand consists of compact masses of fat cells enclosed in a membranous sheath. The cells are formed of big nuclei and homogeneous cytoplasm (Fig. 3A). Malpighian tubules appeared in the section as somewhat rounded tubules, with a one cell layer thick wall and few cells encircling the lumen.The cytoplasm of these cells is filled up with pigmented droplets (Fig. 3B). The ventral nerve ganglion is an oval mass of nerve tissue. The nerve cell bodies are arranged in the lateral ,dorsal and ventral parts. The central part is occuppied by a dense mass of fibrous tissue or the neuropile mass, (Fig. 3C).

b. Treated larvae.

Histological examination of the 3rd instar larvae treated with B. thuringiensis showed that most of the tissues were affected (Fig. 4) Concerning the mid gut tissues , the muscular coat was found to be detached from the epithelial layer. The circuler

and longitudinal muscle fibers were diffcult to be distinguished. The epithelial cells were strongly vacuolated and the boundary lines between the cells disappeared. The nuclei were scattered in a random fashion and most of them were disintigrated (Fig.5) The regenerative cells were hardly seen. The nerve ganglion mass was highly affected. The nerve cells bodies were destructed and many vacuoles appeared in the neuropile mass (Fig. 6A) An increase in the lumen of Malpighian tubules appeared as a result of shrinkage of the cytoplasm of the epithelial cells forming their walls. The epithelial cells showed considerable cytoplasmic vacuolation and some cells were disintegrated (Fig. 6B).

2- Fifth larval instar:

Histological examination of the control 5 th instar larvae of Pieris brassicae showed that it is formed of the same structures of 3rd instar lavae.

The present investigation showed that there are similarities in the histopathological changes induced by B. thuringiensis in both 3rd and 5th larval instars of P. brassicae, (Fig. 7). The most apparent changes in the mid gut was the cytoplasmic vacuolation of the columnar cells and destruction of their nuclei. The musculosa was detached from the epithelium. The Malpighian tubule cells became vacuolized and degenerated, (Fig. 8). Nerve ganglion was found to have many vacuoles and appeared as holes within the nerve fibers portion.

DISCUSSION

Results of the present work showed that treating larvae of Pieris brassicae with the bacterial pathogen Bacillus thuringiensis resulted in appearance of many external symptoms. Similar symptoms were recorded in the larvae of Masalia albida under the effect of B. thuringiensis (Bahareth and Sakr, 1994). Habib et al. (1986). reported that general paralysis was the earliest symptoms appearing in the 5 th instar larvae of Brassolis sophorae treated with B. thuringeinsis . Brounbridge and Onyango (1992)

found that a sublethal dose of commercial formulation of B. thuringiensis inhhibited the general activity of the larvae, reduced the rate of larvae weight gain, legnthened the larval developmental period and reduced the rate of pupation in treated Chilo Partellus compared with the untreated controls.

Histological examination of larvae exposed to B. thuringiensis showed that mid gut tissues, Malpighian tubules, and nerve ganglion were the most tissues affected by this pathogen. These changes were somewhat similar to changes reported by many investigators in other insects under the effect of bacterial insecticides. Bahareth and Sakr (1994) found that B. thuringiensis caused histopathological effects in the gut epithelium, Malpighian tubule , nerve ganglion and fat body of Masilia albida larvae. Su(1992) described many morphological changes in the second larval instar of Plutella xylostella infected with B. thuringiensis. Zohdy and Matter (1988) reported that the bacterium B. thuringiensis induced great histopathological changes in the midgut of Culex pipiens larvae when added to water in which they live.

The effect of chemical insecticides on larval tissues attracted the attention of some investigators (Soliman and Soliman (1958) Hamed et al.1974, Metwally et al . 1978, Taha et al. 1991) Soliman and soliman (1958) found that the insecticides, Parathion, DDT, Toxaphene and cotton dust caused histopathological destruction to the tissues of the midgut, muscular system, fat body, Malpighian tubules and nervous system of Prodeni litura. Hamed et al. (1974) reported that dieldrin induced serious effects in the gut epithelium, salivary glands and Malpigian tubules of Anopheles pharoensis larvae. Metwally et al. (1978) mentioned that the tissues of the cotton leaf worm larvae most affected by the organophosphorous insecticides were found to be those of the midgut and nervous system. Taha et al. (1991) observed that the pyrethroid insecticide, fenvalerate, caused histological destruction of the mid gut epithelium, Malpighian tubules and nerve ganglion of the 3 rd instar larvae of Spodoptera littoralis.

The larvicidal effects of B. thuringiensis were reported to be attributed to the presence of more than one toxin. Wolfersberger (1991) found that the process of potassium- phenylalanine cotransport in the midgut brush border membrane of 5th and 6th instar larvae of Lymantria dispar was inhibted by 2 toxins (HD-73 and HD-19) of B. thuringiensis. He added that the inhibitor role of these two toxins was correlated directly with their potency as larvicides. Odou et al. (1991) reported that delta-endotoxins bind to mid gut membranne of Heliothis virscens larvae and this binding to the membranes of the gut epithelial cells is important for the specificity of the bacterial toxin. Black and Snyman (1991) observed that the spore-delta endotoxin complex of B. thuringiensis was effective in control of 2- weeks old Eldana saccharina larvae. In the present work, it is speculated that the histopathological changes recorded in the larvae of Pieris brasssicae may have resulted from one or more toxins of B. thuingiensis.

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EXPLANATION OF FIGURES

Fig. (1). Section in the control 3 rd larval instar of Pieris brassicae, (X 120).

Fig. (2). Enlarged portion of the previous section showing muscle coat (M), Malpighian tubules (MT), columnar cells (C), regenerative cells (R) and lumen (L) of the mid gut, (X 300).

Fig. (3). Enlarged portion of fat body (FB), Malpighian tubule (MT) and Nerve ganglion (NG) of control 3 rd larva, (X 600).

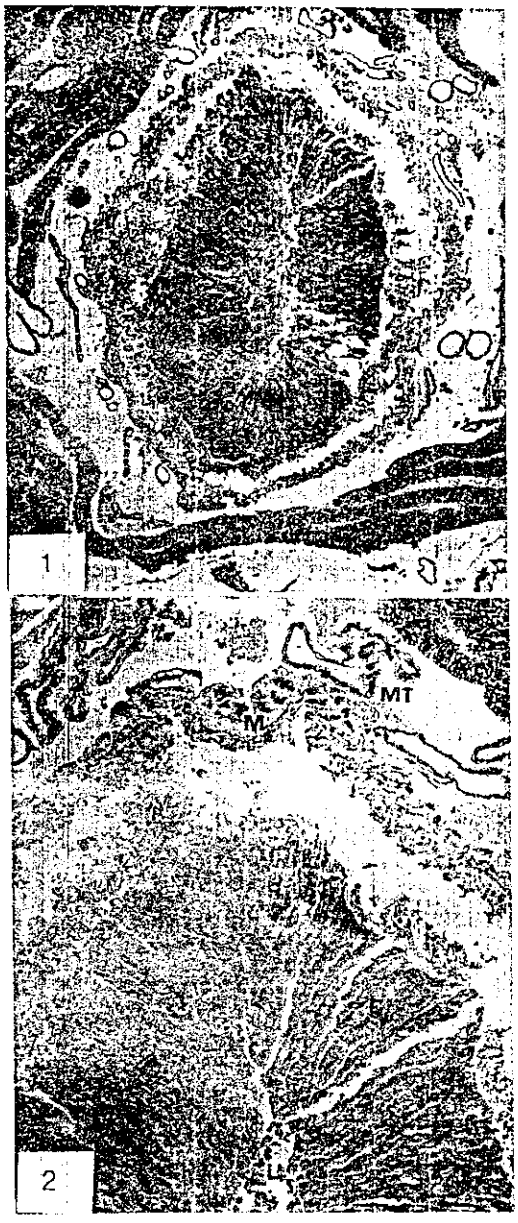
Fig. (4). Section in 3 rd larval instar treated with Bacillus thuringiensis, (X80).

Fig. (5). Enlarged portion of the previous section showing muscle coat (M), epithelial cells (E) with cytoplasmic vacuolation (CV) and lumen (L) of mid gut, (X 300).

Fig. (6). Enlarged portions of the treated larva showing nerve ganglion (NG) and destructed Malpighion tubules, (MT), (X 600).

Fig. (7). Section in the 5th larva treated with B. thuringiensis, (X80).

Fig. (8). Enlarged porthoin of the previous section showing Malpighian tubule (MT), muscle coat (M), vacuolated epithelial cells, (E) and lumen (L) of mid gut, (X300).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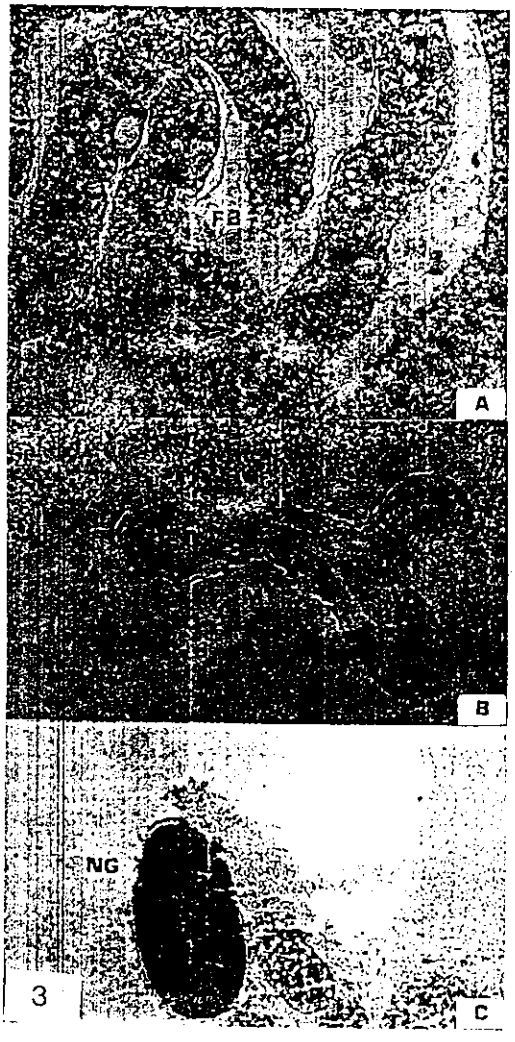
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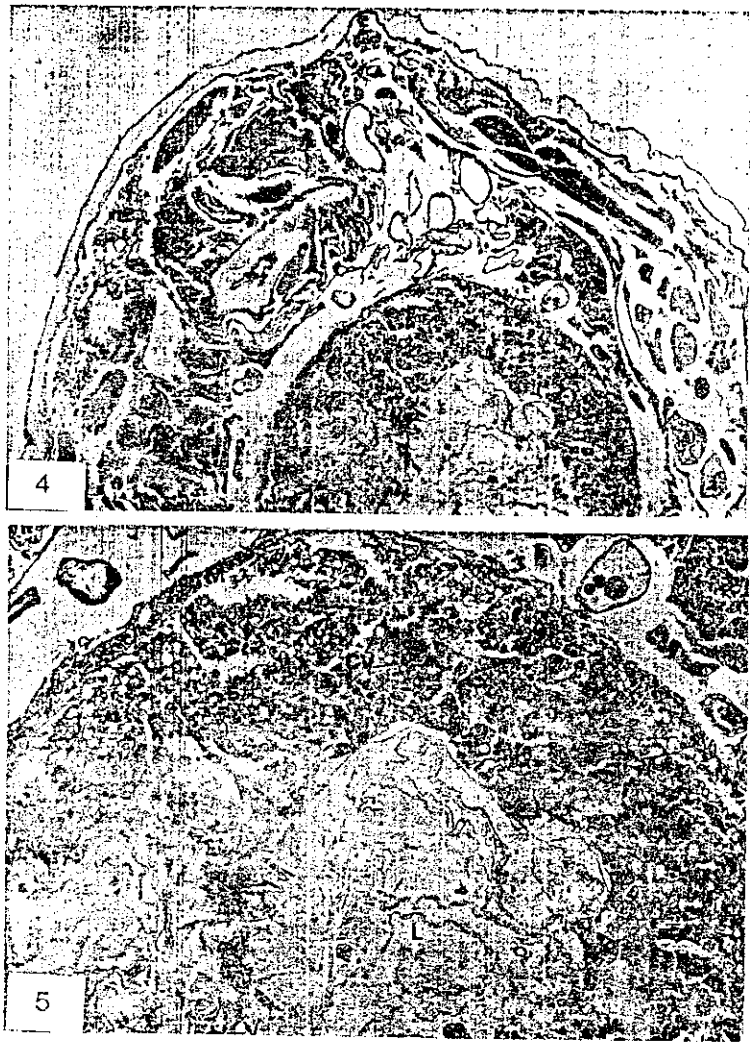
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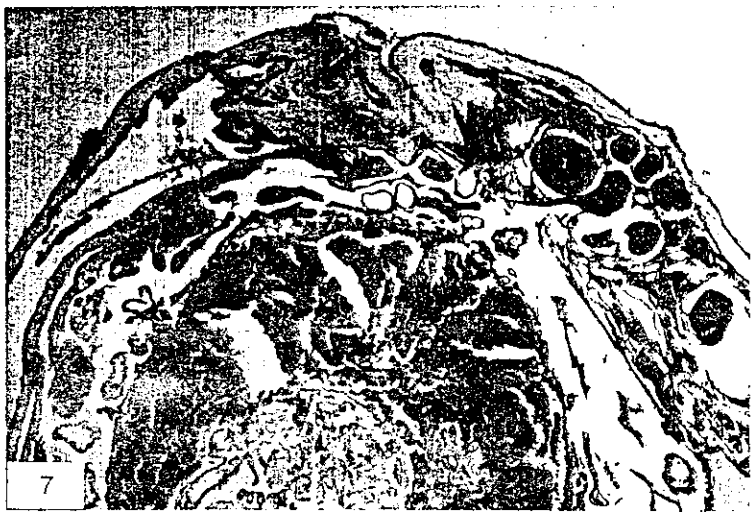
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تأثير المبيد البكتيرى باسيلس ثور نجنسس على التركيب النسيجى ليرقات حشرة أبى دقيق الكرنب بيرس براسيكا

أسامة محمد باحارث صابر عبد صقرالرحمن

قسم الأحياء – كلية العلوم التطبيقية – جامعة أم القرى
مكة المكرمة – المملكة العربية السعودية

درس هذا البحث التركيب النسيجى ليرقات العمر الثالث والخامس لحشرة أبى دقيق الكرنب بيرس براسيكا فى الحالات العادية وتحت تأثير المبيد البكتيرى باسيلس ثور نجنسس .عند تعريض اليرقات للمبيد ظهرت عليها أعراض مرضية خارجية حيث قل نشاطها وأصيب جسمها بالشلل وخرجت السوائل من فمها . أظهر الفحص النسيجى تغيرات هستوباثولوجية فى أنسجة المعى المتوسط، أنابيب ملبيجى والعقد العصبية. ظهرت فجوات سيتوبلازمية فى الخلايا الطلائية للمعى المتوسط وأنابيب ملبيجى وتهدمت أنويتها . تحللت أجسام الخلايا العصبية وظهرت فجوات واسعة فى الجزء الليفى للعقد العصبية .